**Case Report**

Carcinoma with Shared Pathologic Characteristics of Both Hepatocellular Carcinoma and Cholangiocarcinoma

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**ABSTRACT**

**Background:** α-Fetoprotein (AFP) is a useful marker of hepatocellular carcinoma (HCC), and protein induced by vitamin K absence or antagonist II (PIVKA-II) and fucosylated AFP (AFP-L3) are specific tumor markers.

**Objective:** The aim of this article was to report a case of intrahepatic cholangiocarcinoma (CC) with high levels of expression of AFP, AFP-L3, and PIVKA-II.

**Methods:** A 70-year-old man weighing 66 kg with a diagnosis of intrahepatic CC presented with a liver tumor 4.0 cm in diameter and elevated concentrations of carbohydrate antigen 19-9 (575 U/mL), PIVKA-II (379 mAU/mL), and AFP (497 ng/mL; AFP-L3, 88.1%). On extended medial hepatic segmentectomy, microscopy showed that the tumor was a CC without HCC. The patient subsequently underwent immunohistochemical assessments using cytokeratin-19, epithelial membrane antigen (EMA), hepatocyte paraffin-1 (HP-1), PIVKA-II, and AFP.

**Results:** In all specimens, desmoplasia was observed. However, results of immunohistochemistry showed positive results for cytokeratin-19 and EMA; HP-1 results were negative. Results of PIVKA-II and AFP testing in the tumor were positive.

**Conclusions:** The case presented here showed characteristics of CC and HCC, whereas the histologic expression of the tumor suggested CC. Based on the literature search, this is the first known report of a case of a CC expressing AFP and PIVKA-II confirmed on immunohistochemical staining. This case is interesting with regard to the ability of the progenitor cells to differentiate HCC.

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**Key words:** vitamin K absence or antagonist II, fucosylated α-fetoprotein, combined hepatocellular carcinoma–cholangiocarcinoma, hepatocellular carcinoma.

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**INTRODUCTION**

α-Fetoprotein (AFP) is a fetal serum protein produced mainly in the fetal liver and yolk sac, and is a useful marker for hepatocellular carcinoma (HCC). Fucosylated AFP (L3-lectin binding AFP [AFP-L3]) and protein induced by vitamin K absence or antagonist II (PIVKA-II) are specific markers for HCC. Aoyagi et al found that specificity of AFP-L3 for HCC was 86%, and Kuromatsu et al found that it was 92.8% for PIVKA-II.

A combined HCC–cholangiocarcinoma (CC) has histologic and clinical characteristics. However, based on a literature search of MEDLINE (key terms: PIVKA-II, des-gamma-carboxy prothrombin, and cholangiocarcinoma; years: 1993–2005), there have been no reports of a CC without a component of HCC producing AFP and PIVKA-II.

The aim of this article was to describe a patient with a primary intrahepatic CC and high levels of AFP-L3 and PIVKA-II, in whom we found AFP and PIVKA-II localized in tumor cells on immunohistochemical staining of resected tumor.

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**CASE PRESENTATION**

A 70-year-old Japanese man weighing 66 kg presented to his primary care practitioner with epigastralgia and loss of appetite on August 5, 2003. Although he had no history of alcohol dependency, liver function test results from his primary care physician showed elevated serum alanine and aspartate aminotransferases, alkaline phosphatase, total bilirubin, and γ-glutamyl transpeptidase concentrations. Sonography and computed tomography (CT) showed stones in the common bile duct and an intrahepatic tumor (4th segment). After 4 days of non-invasive treatment with an antibiotic, the patient’s total bilirubin concentration was decreased to near normal, and the epigastralgia had resolved. However, the patient was admitted to Ehime University Hospital, Ehime, Japan, on August 9 for additional investigation of the intrahepatic tumor and endoscopic removal of the common bile duct stones.

Physical examination revealed no abnormalities. However, his concentrations of serum AFP (497 ng/mL [normal value (NV), <20 ng/mL]), ratio of AFP-L3 (88.1% [NV, <15%]), plasma PIVKA-II (379 mAU/mL [NV, <40 mAU/mL]), serum carcinoembryonic antigen (17 ng/mL [NV, <5 ng/mL]), and serum carbohydrate antigen 19-9 (CA 19-9) (575 U/mL [NV, <37 U/mL]) were all increased. Hepatitis viral markers were not detected (Table). A common bile duct stone was found on endoscopic retrograde cholangiopancreatography on August 21 and was removed endoscopically. At that time, contrast-enhanced sonography showed...
Table. Laboratory data on admission.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Parameter</th>
<th>Value</th>
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<tbody>
<tr>
<td>WBCs</td>
<td>7100 cells/μL</td>
<td>ALP</td>
<td>893 IU/L</td>
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<td>Neutrophils</td>
<td>70.2%</td>
<td>γ-GTP</td>
<td>754 IU/L</td>
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<td>Basophils</td>
<td>0.7%</td>
<td>LAP</td>
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<td>Eosinophils</td>
<td>1.7%</td>
<td>ChE</td>
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<tr>
<td>Lymphocytes</td>
<td>18.7%</td>
<td>ZTT</td>
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<tr>
<td>Monocytes</td>
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<td>TTT</td>
<td>1 U</td>
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<tr>
<td>RBCs</td>
<td>400 × 10^4 cells/μL</td>
<td>TC</td>
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<tr>
<td>Hemoglobin</td>
<td>13.3 g/dL</td>
<td>TG</td>
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<td>Hematocrit</td>
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<td>IgG</td>
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<td>Platelets</td>
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<td>PT</td>
<td>81.3%</td>
<td>IgM</td>
<td>357 mg/dL</td>
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<td>135 mEq/L</td>
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<tr>
<td>ESR</td>
<td>18/43 mm</td>
<td>Cl</td>
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<tr>
<td>AFP</td>
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<td>BUN</td>
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<td>AFP-L3</td>
<td>88.1%</td>
<td>Creatinine</td>
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<tr>
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<tr>
<td>CEA</td>
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<td>Ferritin</td>
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<td>CA 19-9</td>
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<td>CRP</td>
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<td>HBcAb</td>
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<tr>
<td>LDH</td>
<td>163 IU/L</td>
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</table>

WBCs = white blood cells; RBCs = red blood cells; PT = prothrombin time; ESR = erythrocyte sedimentation rate; AFP = α-fetoprotein; AFP-L3 = α-fetoprotein L3; PIVKA-II = protein induced by vitamin K absence or antagonism II; CEA = carcinoembryonic antigen; CA 19-9 = carbohydrate antigen 19-9; TP = total protein; D-Bil = direct bilirubin; AST = aspartate aminotransferase; ALT = alanine aminotransferase; LDH = lactate dehydrogenase; ALP = alkaline phosphatase; γ-GTP = γ-guanosine triphosphate; LAP = leukocyte alkaline phosphatase; ChE = cholinesterase; ZTT = thymol turbidity test; TTT = thymol turbidity test; TC = total cholesterol; TG = triglycerides; Ig = immunoglobulin; BUN = blood urea nitrogen; CRP = C-reactive protein; HBAlc = glycosylated hemoglobin; FPG = fasting plasma glucose; HCV Ab = hepatitis C virus antibody; HBsAg = hepatitis B surface antigen; HBcAb = hepatitis B core antibody.

the tumor enhanced gradually from the outside, which has been described as a defect in the late phase. Dynamic CT, superparamagnetic iron oxide magnetic resonance imaging, CT angiography (CTA), and CTA portography (CTAP) were performed. Tumor staining was observed on angiography, and CTA showed a gradual enhancement from the outside of the tumor, which was determined to be the result of a defect in CTAP (Figure 1).
Figure 1. (A) Computed tomographic angiography (CTA) showing the tumor enhanced gradually from the outside and nontypical findings for a hepatocellular carcinoma. (B) CTA portography showing a defect consistent with the tumor.

Extended medial segmentectomy of the liver and cholecystectomy performed on September 16 showed direct invasion of the colon transversum and coincidental resection of the mass. Metastasis of the lymph nodes was not detected, and no other intrahepatic tumors were found during the surgery or on intraoperative sonography. Macroscopy of the resected specimen revealed that the tumor measured $4.6 \times 4.3$ cm, with no capsular formation. All specimens of resected hepatic tumor obtained during the surgery were immediately fixed in 10% formalin, cut on the largest surface, grossly observed, embedded in paraffin, and stained with hematoxylin-eosin and periodic acid-Schiff. Microphotography revealed marked desmoplasia (Figure 2). In addition, the expression and localization of cytokeratin-19, as well as epithelial membrane antigen (EMA) (specific antibodies for cholangiocytes$^{10,11}$) and hepatocyte paraffin-1 (HP-1) (a specific antibody for hepatocytes$^{12}$), were found on immunohistochemistry. Furthermore, expression of AFP and PIVKA-II was found on immunohistochemistry using an antibody-labeled streptavidin-biotin method.$^{13,14}$
Figure 2. Microphotography of the resected specimen revealed the tumor to be an adenocarcinoma with marked fibrous stroma. (Hematoxylin and eosin; magnification ×40 [A] and ×100 [B].)

The presence of serum hepatitis B surface antigen (HBsAg), hepatitis B core antibody, and hepatitis C virus (HCV) antibody was assessed using enzyme-linked immunoassay kits (AxSYM HBsAg, Dainabot, Tokyo, Japan; Architect anti-HBc, Abbott Japan, Matsudo, Japan; and Imcheck-F-HCV, Kokusai-shinyaku, Kobe, Japan, respectively).

All specimens were fixed in formalin. Hepatic immunostaining for PIVKA-II (Eisai Company Ltd., Tokyo Japan), AFP (Nichirei, Tokyo, Japan), EMA (Dako Japan Company, Ltd., Kyoto, Japan), HP-1 (Dako Japan Company, Ltd.), and cytokeratin-19 (Immunotech, Marseille, France) was performed using an antibody-labeled streptavidin-biotin method.

RESULTS
Results of cytokeratin-19 and EMA testing were positive in all of the specimens of the tumor (Figures 3A and 4A), whereas results of HP-1 testing were negative (Figure 4B). The appearance was different from that of the scirrhous pattern of an HCC. As a result, we diagnosed the tumor as a moderate differentiated type of CC, with invasion to the colon submucosa, portal vein lymphoduct, and vein. The tumor was not a scirrhous type of HCC and thought to be differentiated
Figure 3. (A) Cytokeratin-19 testing was positive in all specimens, with positive cells stained (diaminobenzidine [DAB]; magnification × 40). (B) Immunostaining for α-fetoprotein, shown diffusely stained (DAB; magnification × 40). (C) Immunostaining for protein induced by vitamin K absence or antagonism II, which was faintly expressed and stained (DAB; magnification × 40). All staining was performed on mirror specimens.

from cholangiocytes. Furthermore, the tumor specimens expressed AFP and PIVKA-II (Figures 3B and 3C).

DISCUSSION
As mentioned previously, AFP is a marker of HCC, and AFP-L31,2 and PIVKA-II3-5 are specific tumor markers for HCC. PIVKA-II expression is increased in vitamin K–deficient patients, those receiving a vitamin K antagonist, and those with HCC.15

In the patient presented here, jaundice was not apparent at admission to Ehime University Hospital, although high levels of PIVKA-II continued and we did not detect the markers of a typical HCC in any of the resected tumor specimens. Based on the literature search, there have been no reports of a CC producing AFP and PIVKA-II. We found elevated levels of PIVKA-II, AFP-L3, carcinoembryonic
antigen, and CA 19-9, whereas findings on dynamic CT and contrast-enhanced sonography showed nonidentical enhancements in the early vascular phase and a defect in the portal phase such as a typical HCC. Therefore, we initially suspected a combined HCC-CC, which has histologic features of both HCC and CC. Kojiro classified combined HCC-CC into 3 types macroscopically and histologically: (1) HCC and CC are clearly distinguished; (2) HCC and CC are contiguous and share transitional features; and (3) cancer cells can be assessed as either HCC or CC and are considered to be an intermediate type. The histologic appearance of the tumor in the case presented here differed from a typical combined HCC-CC in that the morphologic features were typical of a moderately differentiated CC. On immunohistochemical staining for AFP and PIVKA-II, we observed double-staining with cytokeratin-19 and EMA in serial slices. On immunochemical assessment, cytokeratin-19 and EMA, which are expressed in CC cells, were detected. Thus, despite the typical CC features on histology, the association of both HCC and CC characteristics might present a new concept for recognizing combined HCC-CC.
Regarding the etiology of combined HCC-CC, Goodman et al\textsuperscript{7} noted that neoplastic progenitor cells with the ability to differentiate bidirectionally to HCC and CC might develop into both malignant cell types. Theise et al\textsuperscript{17} reported hepatic stem cell malignancies in 4 adults. As for other malignant tumors producing AFP and PIVKA-II, a few cases of gastric cancer without typical HCC and combined HCC-CC have been reported.\textsuperscript{18,19} The ability of those malignancies to produce serum protein was considered to be due to the strong similarity to hepatic cells.\textsuperscript{20} Ishikura et al\textsuperscript{21,22} speculated the reason to be that the liver is derived from the foregut part of the stomach, and that hepatocytes and cholangiocytes are thought to be derived from the same stem cells. Thus, regarding differentiation of hepatocytes and bile duct cells, a new classification of combined HCC-CC should be added to that proposed by Kojiro\textsuperscript{16} pending the identification of other cases.

CONCLUSIONS
The case presented here showed characteristics of CC and HCC, whereas the histologic expression of the tumor suggested CC. Based on the literature search, this is the first known report of a case of a CC expressing AFP and PIVKA-II confirmed on immunohistochemical staining. This case is interesting with regard to the ability of the progenitor cells to differentiate HCC and CC.

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REFERENCES

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